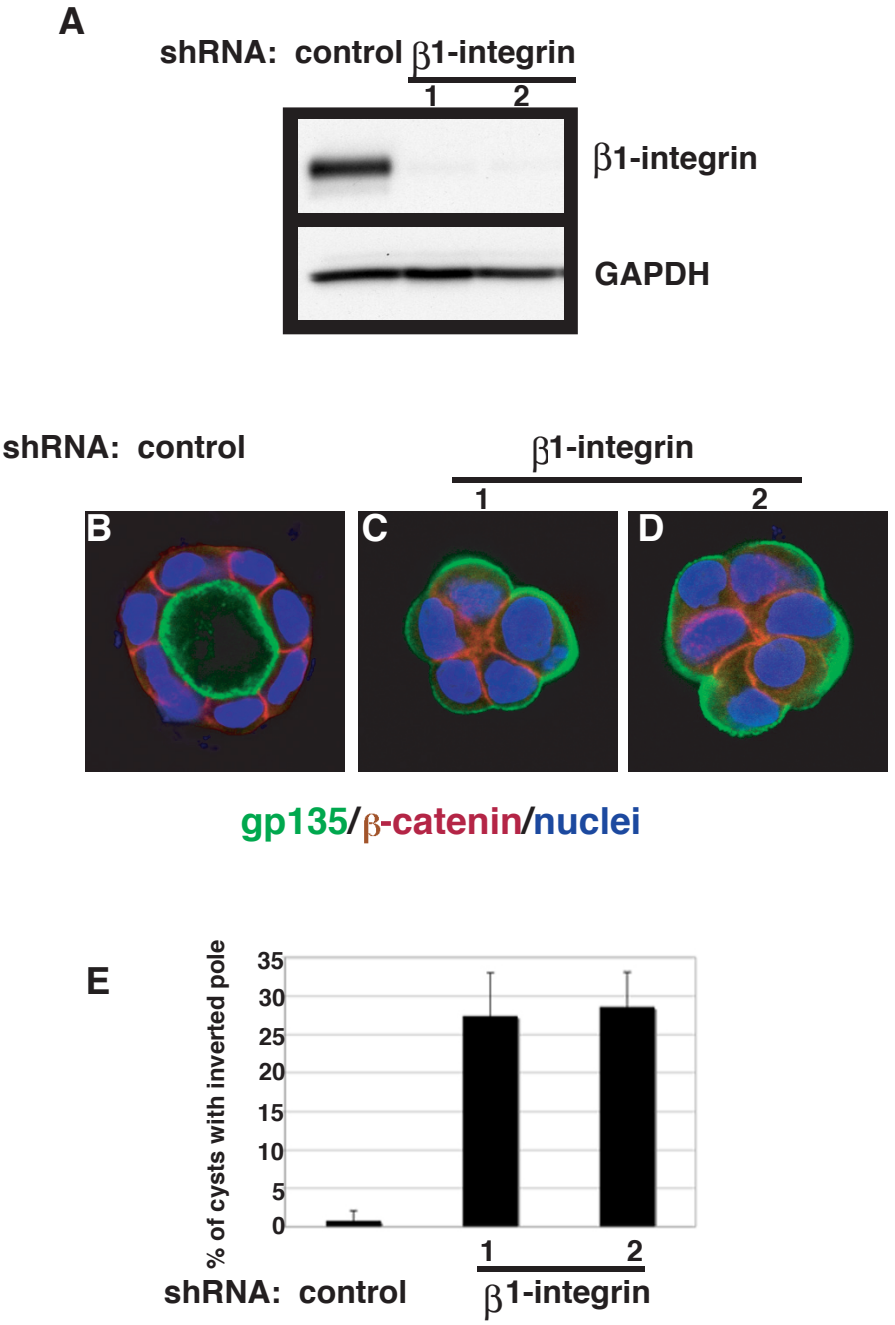
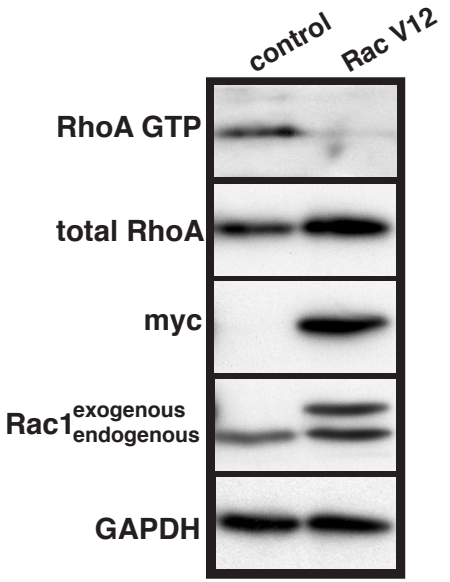
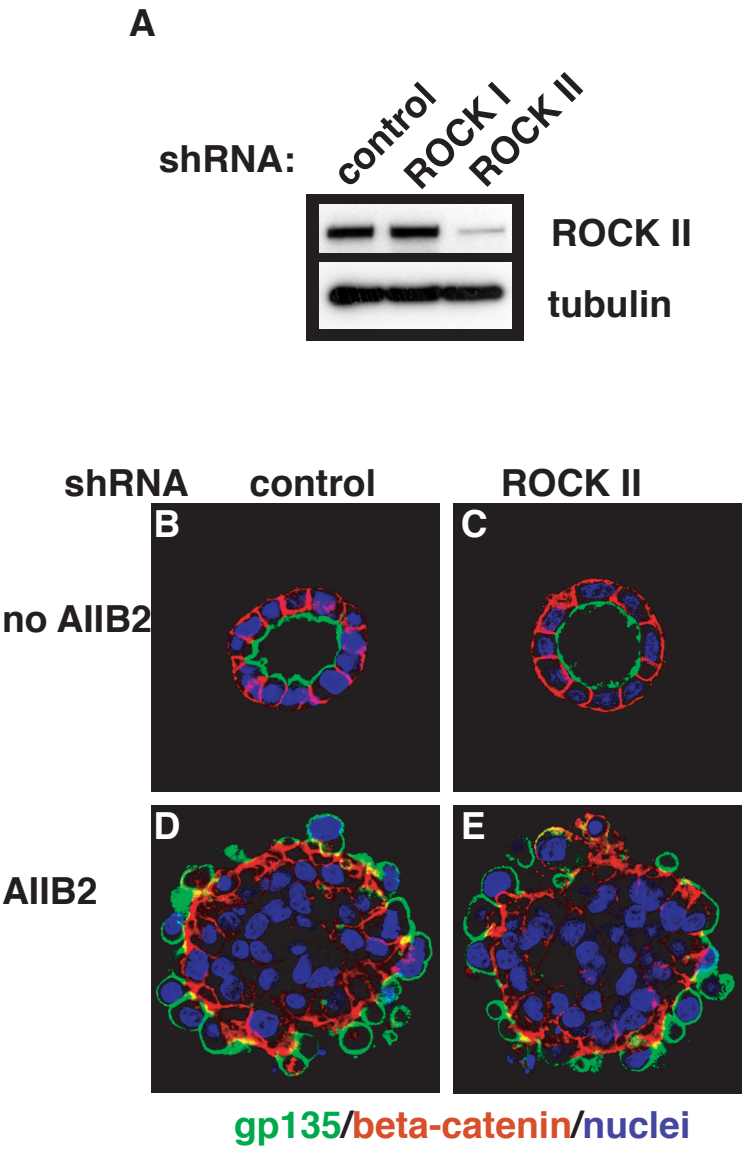


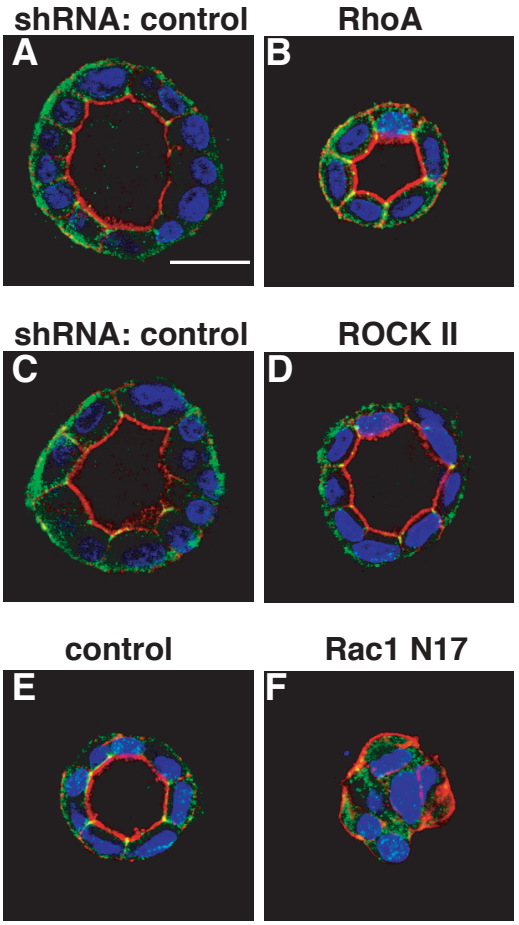
Supplementary figure 1. Yu et al



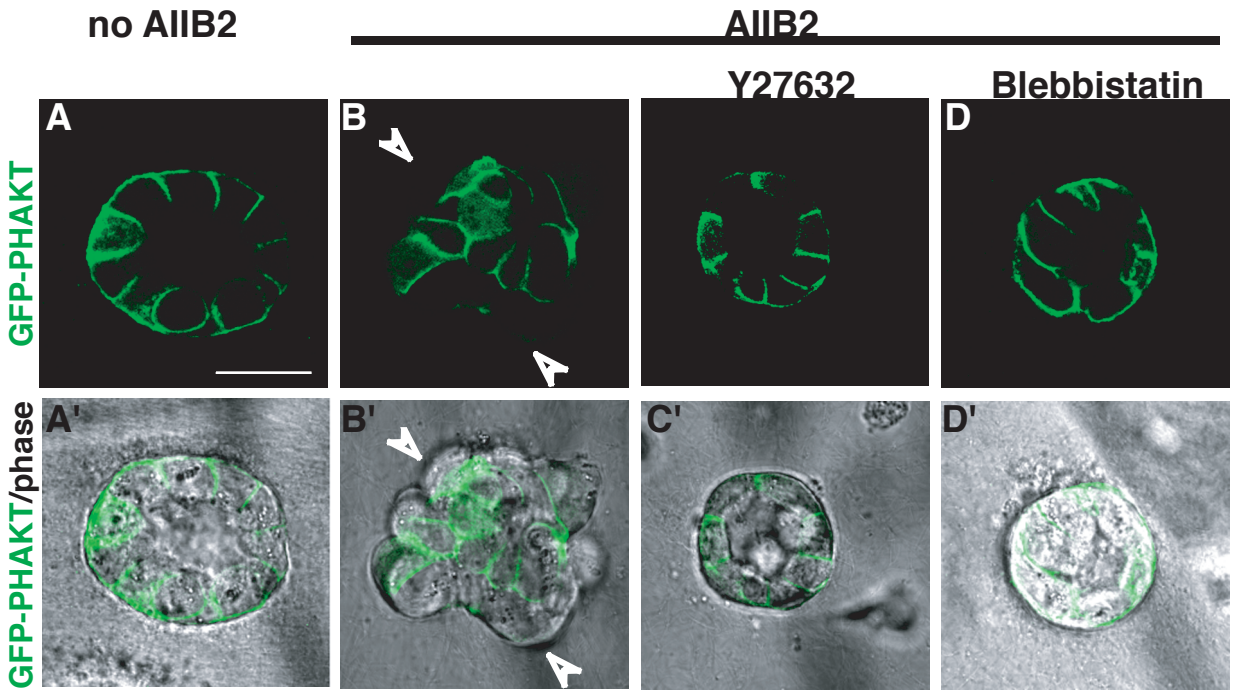
Supplementary figure 2. Yu et al



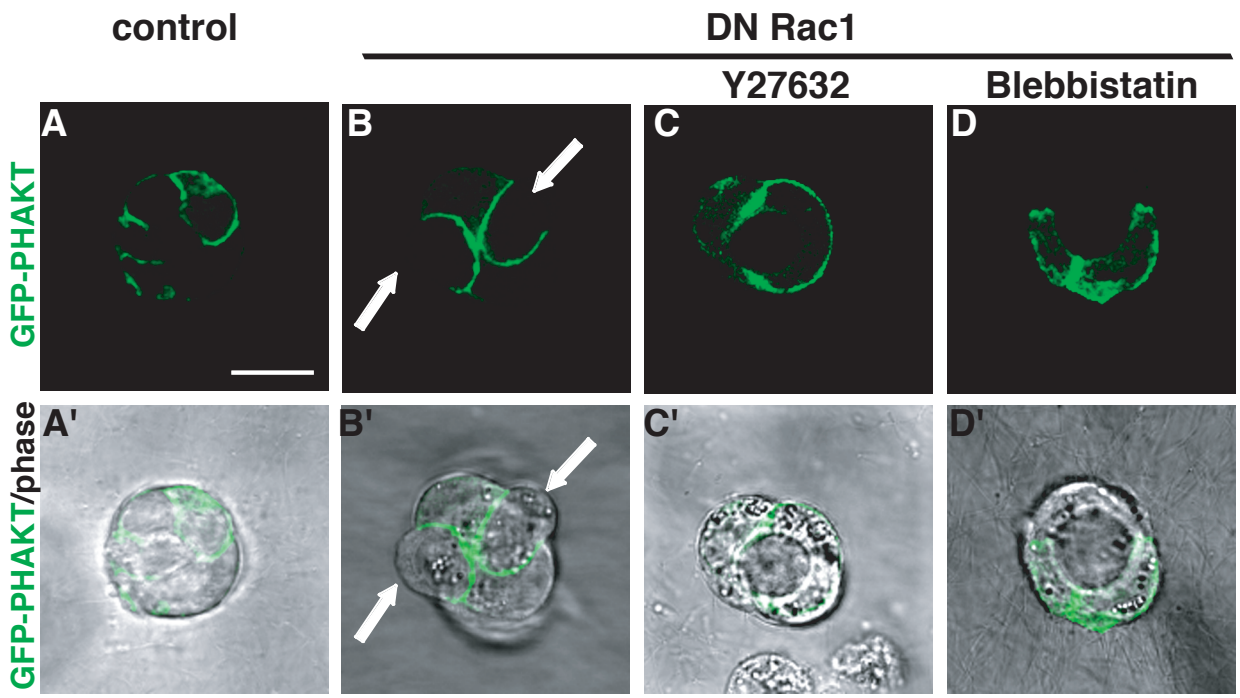
Supplementary figure 3. Yu et al



Supplementary figure 4. Yu et al



Supplementary figure 5. Yu et al



Supplementary figure 6. Yu et al

Target	shRNA sequence
RhoA	CGCCCAUCAUCCUGGUUGGGAA
ROCK I	AAGAAGAACCCUAGAAUCUAC
ROCK II	AGCUGCCUUAUAUGAAUGAG
b1-integrin	1:GAAGGGATGCCATCCAGAT 2:AATCCCAGAGGCTCCAAAGA

Supplementary table Yu et al

Supplementary Material Legends

Figure 1: Knock down of β1-integrin inverts polarity in matrigel culture. Western blot confirms the reduction of β1-integrin by shRNA using the pLKO.1 lentiviral vector (A). Cells grown on Matrigel coated chamber form cysts with inverted polarity as indicated by gp135 (green) at peripheral surface, and β-catenin (red) only at cell-cell contact (B, C comparing control A). (E) The graph represents three experiments.

Figure 2: Activation of Rac1 reduces RhoA activity.
Cells expressing constitutively active Rac1 V12 were embedded in COLI matrix for 24 hours. Exogenous Rac1 expression was confirmed by western blotting for myc and Rac1. RhoA activity (RhoA GTP) was assessed by GST-RBD-Rhotekin pull-down assay and compared to total RhoA.

Figure 3: Knock down of ROCK II doesn't rescue AIIB2 induced polarity. Reduction of ROCK II by RNAi is confirmed by western blot of ROCK II (A). Depletion of ROCK II doesn't prevent polarity inversion in the presence of AIIB2 (E, compare D). Apical marker gp135 in green, basolateral marker β-catenin in red, and nuclei in blue.

Figure 4: Localization of p-MLC in RhoA KD, ROCK II KD and Rac1 N17 cysts.
Loss of function of RhoA and ROCK I by shRNA didn't affect the localization of p-MLC in cysts (compare A to B, C to D). But p-MLC was diffused in cells in Rac1 N17 cyst (F, compare E). Scale bars: 20 μm.

Figure 5: Inhibition of ROCK or myosin II restores PIP3 to BL surface in AIIB2 treated cysts.
(A-D') MDCK cells expressing GFP-tagged PH-Akt, a high affinity marker for PIP3 were plated in COLI to form cysts. Confocal images of live cysts showed GFP-PHAkt, which was normally at BL surface (A and A'), was excluded from peripheral surface in AIIB2 cysts (B and B', white arrow heads). Treatment with Y27632 or blebbistatin restored GFP-PHAkt to the BL surface (C-D"). Scale bars: 20 μm.

Figure 6: Inhibition of ROCK or myosin II restores PIP3 to BL surface in Rac1 N17 cysts.

(A-D') cells expressing Rac1 N17 stably transfected with GFP-tagged PH-Akt were plated in COLI for 3 days to form cysts. Confocal images show GFP- PHAkt, normally at BL surface (A and A'), was excluded from the peripheral surface in Rac1 N17 cysts (B and B', white arrows). Treatment with Y27632 or blebbistatin restored GFP-PHAkt to BL surface (C-D"). Scale bars: 20 μm.

Table: Sequences used for shRNA.